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**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

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**National Food Safety Standard -- In Vitro Mammalian Cells
Chromosome Aberration Test**

食品安全国家标准 体外哺乳细胞染色体畸变试验

(English Translation)

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1 Scope

This standard specifies the in vitro mammalian cell chromosome aberration test basic test methods and technical requirements.

This standard applies to the evaluation of the test substance in vitro mammalian cell chromosome aberration.

2.1 Structural chromosome aberrations

It can be directly observed by a microscope to take place in the cell mitotic metaphase chromosome structural changes. Such as chromosome deletions and Fragment, chromosome swaps. Structural chromosome aberrations can be divided into chromosomal aberrations (chromosome-type aberration) and chromatid Aberrations (chromatid-type aberration).

2.1.1 Chromosome aberrations

Chromosome structural damage, the performance of the two chromatids of the same sites all appear to change broken or broken restructuring.

2.1.2 Chromatid aberrations

Structural chromosome damage, manifested as chromatid breaks or chromatid breakage restructuring and other changes.

2.2 Mitotic index

The number of cells and the medium-term phase ratio of the total number of cells observed; reflect the degree of proliferation cell index.

2.3 Endoreduplication

After the S phase of DNA replication, mitosis starts another S phase nuclei process was not performed. As a result, chromosome There 4,8,16 times chromatids.

2.4 Crack

The length of the chromosome or chromatid damage is less than the width of a chromatid, arranged in a minimum error chromatids.

3 Test purposes and principles

By detecting whether a test substance induced in vitro mammalian cell chromosome aberration, evaluate the possibility of the test substance mutagenic. In Canada Under the system, with or without metabolic activation conditions, the culture of mammalian cells are exposed to the test substance. With metaphase blocker (such as autumn Colchicine or amine) process, the cells stopped at metaphase, and then the cells were harvested, production, dyeing, chromosome aberration analysis.

4.1 Instrument

Incubator, inverted microscope, ultra-clean table, centrifuges.

4.2 Broth

Common Eagle's MEM broth (minimum essential medium, MEM), can also be used other suitable medium. Join Antibiotics (penicillin 100IU/mL, streptomycin 100µg/mL), inactivated fetal bovine serum or calf serum added in a proportion of 10% Broth.

4.3.1.1 Magnesium potassium solution

1.9g of magnesium chloride and potassium chloride 6.15g add distilled water to 100mL.

Disodium hydrogen phosphate (Na_2HPO_4 , 28.4g/L) 440mL, sodium dihydrogen phosphate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 27.6g/L) 60mL, pH adjusted 7.4, 0.103MPa 20min sterilization or to filter bacteria.

4.3.1.3 Coenzyme -II (oxidized) solution

He said under sterile conditions to take coenzyme -II, with sterile distilled water formulated to 0.025mol/L solution using now.

4.3.1.4 Glucose-6-phosphate sodium salt solution

Glucose-6-phosphate sodium salt was weighed, dissolved in distilled water formulated as 0.05mol/L, sterilized by filtration. Using now.

4.3.2 Induced rat liver S9 components and formulation

Choose healthy male adult SD or Wistar rats weighing 150g ~ 200g, about 5 weeks to 6 weeks of age. The PCBs (Aroclor1254) was dissolved in corn oil at a concentration of 200g/L, according to 500mg/kg body weight by intraperitoneal injection once aseptically, 5d after death Animals were sacrificed before fasting 12h.

It may also be used phenobarbital and beta- naphthalene flavones and induction methods for preparing orally administered to rats fed sodium phenobarbital and beta- naphthalene Flavonoids, doses were 80mg/kg body weight, continuous 3d, 16h after fasting animals were decapitated. Other operating with PCBs induction.

Animals were sacrificed after the liver was removed, weighed fresh ice-cold 0.15mol/L potassium chloride solution was continuously flushed several times the liver, can be suppressed in order to remove System microsomal enzyme activity of hemoglobin. Per gram of liver (wet weight) plus 0.1mol/L potassium chloride solution 3mL, along with an ice bath and transferred to a beaker with a sterile Scissors minced liver, in a glass homogenizer (less than 4000r/min, 1min ~ 2min) or tissue homogenizer (less than 20000r/min, 1min)

Made in liver homogenates. The above operation should pay attention to the local cold and sterile environment.

Aliquot in sterile freezer tubes, each tube about 2mL, the best home after liquid nitrogen or dry ice frozen -80 °C cryopreservation.

After S9 fraction prepared by the sterility test, measuring protein content (Lowry method) per milliliter protein content of not more than 40mg is appropriate, and by Indirect mutagens qualified to identify its biological activity after storage at -80 °C low temperature or freeze dry, a period not exceeding one year.

Preparation 4.3.3 10% S9 mixture Usually by the S9 fraction and cofactors press 1.9 mixture consisting of 10% S9, sterile using now. 10% S9 mixture 10mL Prepared as follows.

Take the above phosphate buffer 6.0mL, magnesium potassium solution 0.4mL, glucose-6-phosphate salt solution 1.0mL, coenzyme -II solution 1.6mL, Liver S9 fraction 1.0mL, mix, set in an ice bath until ready to use.

S9 mixture concentration is generally 1% to 10%, the actual concentration determined by individual laboratories, but its activity needs identified, We must be able to significantly activate the positive control, and cells without significant toxicity.

4.4 Colchicine solution

PBS solution with an appropriate concentration of formulation stock solution, filter sterilized, refrigerated in the dark condition can be stored for at least 6 months.

5.59g KCl add distilled water to 1000mL.

4.6 Fixative

Methanol. acetic acid as 3.1, prepared before use. According to the experimental conditions, the concentration of glacial acetic acid can be adjusted to improve the dispersion of chromosomes Degrees, but not too large, leading to cell rupture.

Take Giemsa dye 3.8g, set in a mortar, add a small amount of methanol grinding. Gradually add methanol to 375mL, until completely dissolved, plus 125mL glycerin, into 37 °C incubator insulation 48h. During the incubation shaking several times to fully dissolve. Remove the filter, two weeks after use, as as Giemsa stock solution. When in use, take 1 part Giemsa stock solution was mixed with 9 parts 1/15mol/L phosphate buffer (pH6.8), its application was dubbed, now with the current.

Phosphate buffer (1/15mol/L, pH6.8) formulated as follows.

a) First solution. Take disodium hydrogen phosphate (Na_2HPO_4) 9.47g was dissolved in 1000mL of deionized water, dubbed 1/15mol/L solution;

b) Second solution. take potassium dihydrogen phosphate (KH_2PO_4) 9.07g was dissolved